Evaluation of proniosomes as an alternative strategy to optimize piroxicam transdermal delivery

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Abstract
The current investigation aims to evaluate the transdermal potential of niosomes bearing a potent non-steroidal anti-inflammatory, piroxicam. Piroxicam-loaded niosomes were prepared and characterized for surface morphology, entrapment efficiency and in vitro permeation across excised rat skin from various proniosome gel formulations using Franz diffusion cells. Various non-ionic surfactants were used to achieve optimum encapsulation efficiency. The prepared proniosomes significantly improved drug permeation and reduced the lag time \((p < 0.05)\). Proniosomes prepared with Span 60 provided a higher piroxicam flux across the skin than did those prepared with Tween 80. Niosomes prepared using Span 60 showed a higher release rate than those prepared using non-ionic surfactants, Span 20 and Span 80, while those prepared from Tween showed higher release rate than formula prepared with Span. This indicates that lipophilicity and hydrophilicity of surfactant has a main role in release rates of piroxicam. Particle size of piroxicam niosomal vesicles formed by proniosome was determined by scanning electron microscopy. The encapsulation efficiency was evaluated by a specific high performance liquid chromatography method. Niosomes formed from using Spans and Tweens exhibited very high encapsulation efficiency. The results are very encouraging and suggest that niosomes can act as promising carriers offering an alternative approach for transdermal delivery of piroxicam.

Key words: Niosomes, proniosomes, piroxicam, transdermal delivery, permeation

Introduction
In recent years, the development of transdermal dosage forms has been attracting increasing attention, owing to the several advantages that this administration route offers. Transdermal delivery systems, when compared with conventional formulations, generally show a better control of blood levels, a reduced incidence of systemic toxicity, no hepatic first-pass metabolism and a higher compliance

A continuous interest toward the dermal and transdermal products can be seen, offering several advantages. Data from the US shows that out of 129 drug delivery candidates, 51 transdermal or dermal systems are listed; and 30% of 77 candidate products in pre-clinical development represent such drug delivery system.

Liposomes have attracted a great deal of attention in the delivery drugs because of many advantages: they are biodegradable, non-toxic, amphiphilic in nature, penetration enhancers and effective in the modulation of drug release properties. Although the application of liposomes for improved drug delivery is encouraging, liposomes exhibits some difficulties, including the instability of aqueous dispersions on storage and the leakage of the encapsulated drugs. Moreover, the high cost of synthetic phospholipids and variable purity of natural phospholipids have raised concerns over the adoption of liposomal drug delivery systems.

An alternative approach that overcomes several of these problems associated with liposomes involves formation of liposome-like vesicles (niosomal dispersions) from non-ionic surfactants, commonly referred as to as...
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Niosomes7,8 Niosomes are capable of entrapping hydrophilic and hydrophobic solutes8,10. The greater stability, lesser storage problems and lack of many disadvantages associated with liposomes, such as high cost and the variable purity problems of phospholipids have prompted the exploitation of niosomes as alternative promising drug carriers to phospholipids vesicles for industrial production of both pharmaceutical and cosmetic products13–14. Niosomes can entrap solutes, are quite stable, and require no special conditions, such as low temperature or inert atmosphere for production or storage9.

Niosomes and proniosomes, hydrated by agitation in hot water for a short period of time, have been proposed for a number of potential therapeutic applications, i.e. as carriers of anti-inflammatory drugs15 and as anti-infective drug targeting agents16. In addition, niosomes are versatile carrier systems that can be administered through various routes17. Particular efforts have been aimed at using niosomes as effective transdermal drug delivery systems13,9,18,19.

Piroxicam (PC) is one of the most potent non-steroidal anti-inflammatory agents that also has anti-pyretic activity and has been used for the treatment of rheumatoid arthritis, osteoarthritis and traumatic contusions20. PC has been classified in the Biopharmaceutics Drug Classification system as a Class II drug with low solubility and high permeability. It demonstrates a slow and gradual absorption via the oral route and has a long half-life of elimination, rendering a prolonged therapeutic action and a delayed onset of anti-inflammatory and analgesic effect21. PC is well absorbed following oral administration; however, its use has been limited by a number of side effects, including bleeding and ulceration22. Therefore, an alternative non-invasive mode of delivery of the drug is needed. Transdermal administration of piroxicam can overcome these side effects and higher local concentration can be maintained at the target site, which is desirable for anti-inflammatory agents23. The transdermal drug delivery system appears to be an attractive route of administration to maintain the drug blood levels of PC for an extended period24.

Several research attempts have been made to enhance release of PC using different formulation strategies, including inclusion complexation with β-cyclodextrin20,25, iontophoresis technique26 as well as the use of poloxamer gel27; however, most of these efforts require sophistication techniques and the encapsulation efficiency obtained was very low (i.e. 12.73)%28,27,26,25. In addition, nobody has investigated the feasibility of using proniosomes as a potential carrier for transdermal delivery of PC.

The objectives of this study were to develop a transdermal delivery system of piroxicam using proniosomes and to study its in vitro permeation characteristics.

Materials and methods

Materials

Span 20, 60 and 80 were supplied from Koch-light laboratories Ltd. (Coebrook Bucks, UK). Tween 20, 60 and 80, acetonitrile HPLC grade, potassium dihydrogen phosphate and disodium hydrogen phosphate were purchased from BDH Laboratory Supplies (BDH Chemicals Ltd., Poole, UK). Lecithin from eggs (extra pure) was obtained from Merck Company (Darmstadt, Germany). Cholesterol, acetic acid and ethanol were supplied from Riedel Dehau (Darmstadt, Germany). Hydroxypropyl methylcellulose (HPMC K100 Premium CR grade) was obtained from Dow Chemical Company (Midland, MI). Piroxicam was purchased from Sigma Chemicals Company (St. Louis, MO).

Preparation of proniosomes

The method reported by29 was followed with slight modifications. The method briefly as follows: the drug (100 mg of PC) with surfactant, lecithin and cholesterol were taken in a clean and dry, wide mouth small glass tube and mixed with 2.5 ml of absolute ethanol. After mixing, the open-end of the glass tube was covered with a lid to prevent loss of solvent from it and warmed on a water bath at 65 ± 3 °C for ~5 min, until the surfactants were dissolved completely. Then 1.6 ml of pH 7.4 phosphate buffer was added and the mixture was further warmed in the water bath for ~2 min so that a clear solution was obtained. The mixture was then left to stand at room temperature until the dispersion was converted to proniosomal gel. The proniosomal gel was then mixed with 2% w/w of polymeric gel (HPMC) to give a final concentration of 0.5% w/w PC. A similar manner was used to prepare control (empty niosomal dispersion) proniosomal gel (free of drug). The composition of different proniosomal formulations (mg and mmols) is shown in Table 1.

Piroxicam encapsulation efficiency

The concentration of drug entrapped was determined by taking 0.2 g of proniosome gel, weighed in a glass tube and added to 10 ml of pH 7.4 phosphate buffer. The aqueous suspension was sonicated in a sonicator bath (Transonic T460/H, Elma, Germany). The PC-containing niosomes were separated from untrapped drug by centrifugation at 25 000 rpm (32 000 × g) at 20°C for 30 min. The supernatant was recovered and assayed by an HPLC method.
for PC content. The percentage of drug encapsulation (EE (%)) was calculated by the following equation:

\[
EE(\%) = \left( \frac{C_t - C_f}{C_t} \right) \times 100\%
\]

where \( C_t \) is the concentration of total PC and \( C_f \) is the concentration of free PC.

**Vesicular shape and surface morphology**

Scanning electron microscopy (SEM) was conducted to characterize the surface morphology of the niosomes including the controls (empty vesicles). One drop of niosomal system was mounted on clear glass stub, air dried and sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator (Edwards) and examined using a scanning electron microscope JSM-5510 (Jeol Ltd., Tokyo, Japan) equipped with a digital camera, at 15 or 20 kV accelerating voltage.

**In vitro release study**

To determine drug release, 1 g of proniosomal gel of different compositions was spread on a glass circular disk (5.04 cm² diameter), then covered by cellophane dialysing membrane with molecular weight cut-off of 8000 (Spectrum Medical Inc., Los Angeles, CA) which was securely mounted on the disk by a rubber band. The disk was placed on the bottom of a beaker glass tube large enough to accommodate the disk diameter and 50 ml of pH 7.4 phosphate buffer was poured onto the membrane surface. The whole assembly was immersed in a water bath maintained at 37°C. The buffer solution was continuously circulated over the membrane surface in a closed circle at a rate of 5 ml min⁻¹ using a Watson–Marlow peristaltic pump. The drug release was monitored using an automated monitoring system, which consisted of an IBM computer and PU 8605/60 dissolution software, a Philips Vis/UV/NIR single beam eight cell spectrophotometer model PU 8620. For each formula, drug release was studied in triplicate, absorbance at 354 nm was recorded automatically over 6 h and the percentage of drug release was calculated.

**In vitro permeation study**

This parameter is very important in evaluating the potential use of a delivery system as a topical device. The permeation of PC from proniosomal formulations was investigated by using an *in vitro* Franz diffusion cell. The temperature was maintained at 37°C. The shaved dorsal skin of albino rat (0.4 ± 0.1 mm thickness and 3.14 cm² exposed surface area) was mounted on the receptor compartment with the stratum corneum side facing upwards towards the donor compartment. The receptor compartment was filled with 15.0 ml of pH 7.4 phosphate buffer maintained at 37°C and was constantly stirred by a magnetic stirrer at 600 rpm. Proniosomal gel formulation (1 g) was placed on the skin and the top of the diffusion cell was covered with paraffin paper. Samples (1 ml) were withdrawn through the sampling port of the diffusion cell at pre-determined time intervals (2, 4, 6, 8, 12, 16, 20 and 24 h) and analysed for drug content by HPLC. The receptor medium was immediately replenished with equal volume of fresh diffusion solution. Sink conditions were maintained throughout all the experiment. Triplicate experiments were conducted for each study.

**HPLC analytical method**

To each 0.5 ml sample, 0.5 ml of 2% zinc sulphate solution, a protein precipitant, was added and the mixture vortexed (Scientific Industries, Inc., NY) for 1 min and then centrifuged at 13 000 rpm (22 500 × g) for 10 min. The supernatant was directly injected into the HPLC system. The piroxicam content of these various samples was analysed by a modified literature HPLC method. The HPLC system consisted of a Waters pump Model 1515, Waters autosampler Model 717 plus (Waters Inc., Bedford, MA), a Waters 2487 dual λ UV detector controlled by a

### Table 1. Compositions of various proniosomal formulations in milligrams (mmols).

<table>
<thead>
<tr>
<th>Pronosome code (Formula)</th>
<th>Piroxicam</th>
<th>Span 20 (S20)</th>
<th>Tween 20 (T20)</th>
<th>Lecithin</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Span 20 (S20)</td>
<td>100 (0.3)</td>
<td>1800 (5.20)</td>
<td>1800 (1.46)</td>
<td>1800 (2.73)</td>
<td>200 (0.52)</td>
</tr>
<tr>
<td>Span 60 (S60)</td>
<td>100 (0.3)</td>
<td>1800 (4.18)</td>
<td>1800 (1.37)</td>
<td>1800 (2.73)</td>
<td>200 (0.52)</td>
</tr>
<tr>
<td>Span 80 (S80)</td>
<td>100 (0.3)</td>
<td>1800 (4.20)</td>
<td>1800 (1.37)</td>
<td>1800 (2.73)</td>
<td>200 (0.52)</td>
</tr>
<tr>
<td>Tween 20 (T20)</td>
<td>100 (0.3)</td>
<td>-</td>
<td>1800 (1.46)</td>
<td>1800 (2.73)</td>
<td>200 (0.52)</td>
</tr>
<tr>
<td>Tween 60 (T60)</td>
<td>100 (0.3)</td>
<td>-</td>
<td>1800 (1.37)</td>
<td>1800 (2.73)</td>
<td>200 (0.52)</td>
</tr>
<tr>
<td>Tween 80 (T80)</td>
<td>100 (0.3)</td>
<td>-</td>
<td>1800 (1.37)</td>
<td>1800 (2.73)</td>
<td>200 (0.52)</td>
</tr>
</tbody>
</table>
microcomputer running Empower® Build 1154 software. The detector wavelength was set at 354 nm. Separation was achieved by isocratic elution with a mobile phase consisting of 55:45 v/v water/acetonitrile with the pH adjusted to pH 3.2 using glacial acetic acid, delivered at a flow rate of 1 ml min⁻¹ at ambient temperature through a μ-Bondapack C₁₈ analytical column, 150 × 4.6 mm ID, 5 μm particle size (Waters Inc., Bedford, MA).

**Statistical analysis**

Data were expressed as the mean of three experiments ± the standard deviation (SD) and were analysed using one-way analysis of variance (ANOVA), followed by Scheffe post-hoc tests using SPSS® version 11. p < 0.05 denoted statistical significance.

**Results and discussion**

The objective in developing proniosomes was to devise a method of producing a non-ionic surfactant-based dosage at the point of use to avoid problems of physical and chemical stability found in storage of some surfactant-based dosage forms.

The method of preparation of proniosomes is based on the simple idea that the mixture of surfactant:alcohol:aqueous phase can be used to form the concentrated proniosomal gel, which can spontaneously be converted to a stable niosomal dispersion by dilution with excess aqueous phase³²,¹³.

Piroxicam was successfully encapsulated within the proniosomal gel. HPMC gel was chosen in this study as a suitable polymeric gel vehicle due to the negligible effect on drug release rate from proniosomes when compared to other polymeric gel polymers, sodium carboxymethylcellulose and carbopol 934, which resulted in an enhancement or retardation of drug release rate³³.

**In vitro release study**

Figure 1 shows the cumulative release profile of piroxicam from different proniosomal gel formulations compared to its release from HPMC polymeric gel (control). It is obvious that there is a significant difference between the drug release rates from HPMC and proniosomal gels formulations in the release rate (p < 0.05). This could be explained on the basis of high lipophilicity characteristics of piroxicam which favours more partition in the proniosomal gels. This concept could also explain the lower release rates observed from Span formulations compared to Tween formulations. This indicates that lipophilicity and hydrophilicity of surfactant has a main role in release rates of piroxicam.

Niosomes prepared using Span 60 showed a higher release rate than those prepared using non-ionic surfactants, Span 20 and Span 80 (Table 2). Spans 20 and 80 possess lower phase transition temperatures and form less permeable rigid bilayers than Span 60, which forms a more permeable fluid bilayer (Namaœo and Jain 1999). Similar results were also observed with Tween 80 when compared to Tweens 20 and 60 (Table 2). Based on the in vitro release results, Span 60 and Tween 80 (showing higher release rates) were selected for further studies.

**Encapsulation efficiency**

As shown in Table 3, niosomes formed from using Spans and Tweens exhibited very high encapsulation efficiency. This could be explained on the basis that the highly lipophilic portion of the drug is expected to be housed almost completely within the lipid bilayer of the niosomes. Similar observations have been previously reported³⁴. The results are also consistent with the high entrapment efficiency of levonorgestrel in proniosomes¹³. Most of the

![Figure 1. In vitro release of piroxicam from different polymeric gels and proniosomes.](image)

<table>
<thead>
<tr>
<th>Table 2. Release rate of piroxicam through a cellophane membrane from different formulations.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>S20</td>
</tr>
<tr>
<td>S60</td>
</tr>
<tr>
<td>S80</td>
</tr>
<tr>
<td>T20</td>
</tr>
<tr>
<td>T60</td>
</tr>
<tr>
<td>T80</td>
</tr>
<tr>
<td>Control</td>
</tr>
</tbody>
</table>
surfactants used to make non-ionic surfactant vesicles have a low aqueous solubility. However, freely soluble non-ionic surfactants such as Tweens can form micelles on hydration in the presence of cholesterol\textsuperscript{12}. The Tween formulations in the present study were also able to entrap PC efficiently. The increased entrapment efficiency of proniosomes prepared using Span 60 may be attributed to the increase in the availability of lipophilic ambience, which can accommodate the drug molecules to a higher extent. The highest entrapment efficiency of Span 60 could be attributed to the phase transition temperature\textsuperscript{35}. Span 60 is solid at room temperature and showed the higher phase transition temperature. Thus, Span having the highest phase transition temperature provides the highest entrapment. A similar observation was also noted with the antipsoriatic drug, dithranol\textsuperscript{36}.

**Morphology and vesicle size of proniosomes**

In the presence of cholesterol, all formulations used during this study were able to form stable vesicle dispersions.

Table 3. Characterization of proniosomal formulations by encapsulation and vesicle size after dilution with buffer.\textsuperscript{*}

<table>
<thead>
<tr>
<th>Proniosome code</th>
<th>Encapsulation (%)</th>
<th>Vesicle size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S60</td>
<td>91.7 ± 6.2</td>
<td>4.81 ± 1.1</td>
</tr>
<tr>
<td>Control (Span 60)</td>
<td>-</td>
<td>4.12 ± 1.3</td>
</tr>
<tr>
<td>T80</td>
<td>89.9 ± 4.7</td>
<td>33.2 ± 4.6</td>
</tr>
<tr>
<td>Control (Tween 80)</td>
<td>-</td>
<td>32.1 ± 2.2</td>
</tr>
</tbody>
</table>

*Each value represents the mean ± SD (n = 3).

The scanning electron microscopy images of the niosomes prepared from different proniosomal formulations are shown in Figure 2. Most of the vesicles are spherical and discrete with sharp boundaries. The mean vesicle sizes of the niosomes formed from piroxicam proniosome formulations are presented in Table 3. Niosomes prepared with Tween 80 (Figure 2(c)) were significantly larger than those prepared with Span 60 (Figure 2(a)) (p < 0.05). The relationship observed between niosome size and Span hydrophobicity has been attributed to the decrease in surface energy with increasing hydrophobicity\textsuperscript{35}, resulting in the smaller vesicles. This would also explain the large vesicle size of niosomes prepared with Tween which has a much lower hydrophobicity than does Span. In order to demonstrate if PC has an effect on vesicle size, empty niosomal vesicles (Figures 2(b) and (d)) were prepared using exactly the same methods of preparation in order to obtain an appropriate comparison with niosomal vesicles (prepared with either Span 60 or Tween 80) loaded with PC. The results (Table 3) revealed that the empty (control) niosomes were not statistically (p > 0.05) different from those prepared loaded with PC. This is experimental evidence of incorporation of PC in the lipophilic bilayer of the niosomes.

**In vitro permeation study**

Proniosomes should be hydrated to form niosomal vesicles (Gupta et al. 2007b) before the drug is released and permeates across the skin. Several mechanisms could explain the ability of niosomes to modulate drug transfer
across skin\textsuperscript{12,13,37} including (i) adsorption and fusion of niosomes onto the surface of skin would facilitate drug permeation, (ii) the vesicles act as penetration enhancers to reduce the barrier properties of the stratum corneum and (iii) the lipid bilayers of niosomes act as a rate-limiting membrane barrier for drugs.

Figure 3 depicts the permeation profile of PC across excised rat skin participated with surfactants. Control PC passed through the skin is significantly lower than those proniosomal formulations prepared using non-ionic surfactants, suggesting that the lipid bilayers of niosomes act as a permeation enhancer for PC across rat skin. The mechanisms by which proniosomes permeate across skin such as permeation enhancers effect and vesicle-skin interactions may justify this finding\textsuperscript{38}.

It is clear from Tables 2 and 4 that the release rate of piroxicam across the cellulose membrane is significantly higher than its flux across the skin ($p < 0.05$), indicating the barrier properties of skin for the drug. Since there were great discrepancies between the permeation profiles of the drug proniosomal formulations across skin and across cellulose membrane, interaction between skin and proniosomes may be an important contribution to the improvement of piroxicam transdermal drug delivery.

One of the possible reasons for niosomes to enhance the permeability of drugs is structure modification of the stratum corneum\textsuperscript{38}. It has been reported that the intercellular lipid barrier in the stratum corneum would be dramatically looser and more permeable following treatment with liposomes and niosomes\textsuperscript{39,37}. Both phospholipids and non-ionic surfactants in the proniosomes always act as penetration enhancers, which are useful for increasing the permeation of many drugs. Another explanation was that the niosomes vesicles in contact with stratum corneum aggregated and fused at the interface of the stratum corneum and a high local drug concentration in the bilayers generated a high thermodynamic activity of PC in the upper part of the stratum corneum to the surface of skin, demonstrated in a previous report\textsuperscript{38}, results in higher flux of the drug due to direct transfer of drug from vesicles to the skin.

Proniosomes prepared with Span 60 showed a higher enhancement effect (permeation rate) than those prepared with Tween 80. This was expected due to the smaller size of the vesicles and the higher lipophilic nature of vesicles prepared with Span 60, which makes it easier for these vesicles to penetrate or fuse with the skin. Vesicular system mechanisms have been explored using different vesicles models, soybean phosphatidylcholine (PC) liposomes and Span 60 niosomes to investigate the reasons for permeation enhancement\textsuperscript{19}. It was found that the permeation across Span 60-treated skin was significantly higher than that across soybean PC and non-treated skin. Formulations treated with Span 60 were superior in facilitating the permeation of enoxacin as well as drug deposition into the skin. Span 60 can serve as a permeation enhancer. It is clear from these findings that proniosomal size alone does not determine the drug release profiles and/or permeation processes. The effects seen are related to the high lipophilicity characteristics of piroxicam which favour more partition in the proniosomal gels.

| Table 4. Permeation parameters for piroxicam across excised rat skin. |
|---|---|---|---|---|
| Formula | Cumulative amount (µg cm\textsuperscript{-2}) | Flux (µg cm\textsuperscript{-2} h\textsuperscript{-1}) | Permeability coefficient (cm h\textsuperscript{-1}) | Lag time (h) |
| S60 | 384 ± 8.2 | 16.68 ± 2.9 | 3.33 × 10\textsuperscript{-3} | 0.753 | 4.72 |
| T80 | 306 ± 11 | 13.31 ± 2.8 | 2.66 × 10\textsuperscript{-3} | 0.750 | 3.77 |
| Control | 80 ± 4.5 | 3.53 ± 0.32 | 7.06 × 10\textsuperscript{-4} | 1.025 | 1 |

ER = enhancement ratio, each value represents the mean ± SD (n = 3).

Conclusions

This paper has shown that piroxicam can be entrapped in niosomes with high efficiency using various compositions and types of non-ionic surfactants. Niosomes may provide a means to deliver PC. In addition, the low cost of the surfactants used for preparing niosomes and their greater stability compared with liposomes makes them an attractive alternative. The experimental findings show that either fusion of the vesicles with the intercellular lipid of the stratum corneum and direct transfer of drug from vesicles to the skin and/or the penetration enhancement effect of the non-ionic surfactants may contribute to the mechanism of drug permeation enhancement by
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proniosomal formulations. The present study revealed that PC-loaded proniosomes provided an enhanced transdermal flux, lower lag time and higher entrapment efficiency, thus leading to the generic conclusion that this approach offers a suitable approach for transdermal delivery of PC.

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Declaration of interest: The author reports no conflicts of interest. The author alone is responsible for the content and writing of the paper.

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